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Prevalence of dengue NS1 antigenemia among healthy blood donors in a tertiary care hospital in Southern India

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Abstract:

INTRODUCTION: Transmission of dengue by transfusion of blood products has been documented, although the frequency of these occurrences and the level of viremia required to cause clinical dengue are unknown. The primary objective was to assess the prevalence of dengue NS1 antigen among healthy blood donors at our blood center.

METHODOLOGY: This was a cross-sectional study conducted in the Department of Transfusion Medicine, a tertiary care hospital in South-eastern India, from February 2019 to January 2020. A total of 968 donor samples were included in the study. Dengue NS1 antigen detection was done using enzyme-linked immunosorbent assay. Data regarding clinical, epidemiological, and demographic characteristics were collected from the donor questionnaire and records.

RESULTS: In the study, the overall prevalence of Dengue NS1 antigen was 0.9%, with nine positive samples among the 968 samples tested. Eight of them were male, and 1 was a female donor. All of them were in the age group <32 years. Half of the positive donors were detected during December-January, the immediate post rainy season in this part of the country. Two-third of the positive donors were from rural areas.

CONCLUSION: This study with a 0.9% throws light on the seroepidemiological prevalence of dengue among asymptomatic donors and gives an insight into whether dengue screening is required to be implemented in routine transfusion transmissible infection screening in blood transfusion services and shall assist in devising strategies to be adapted as to improve the blood safety.

Keywords:

Blood donors, NS1 antigenemia, Southern India

Introduction

Emerging infections are seen as a growing danger among the human population, and those that are transmitted by blood transfusions are of particular interest and essential for transfusion services. In a developing nation like India, dengue transmitted via blood transfusion is still not a problem to reckon with, probably because of lack of awareness regarding such a route of transmission of dengue infection or the gravity and impact of the same in health

care. Transmission of dengue by transfusion of blood products has been documented, although the frequency of these occurrences and the level of viremia required to cause clinical dengue is unknown.^[1]

Dengue has a viremic phase of 4–8 days, with most infections falling under the subclinical category. However, in this viremic phase, both symptomatic and asymptomatic individuals can act as potential vehicles for dengue transmission when the said individuals undertake blood donation.^[2] We included Dengue NS1 antigen for detection in our donors instead of immunoglobulin M (IgM) and IgG as

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antigenemia is implicated in transfusion-transmitted dengue, whereas IgM/IgG, which is usually formed much later in the course of infection and therefore, is preferred when donors are symptomatic. Furthermore, the NS1 antigen is found to be more stable in blood when compared to DENV RNA.^[3]

This study was designed to determine the prevalence of Dengue NS1 antigenemia in healthy blood donors in a tertiary care center in Southern India. Studies from this part of the country are sparse on NS1 Antigenemia, and the available study was done on a very small sample to draw a meaningful conclusion if any.^[4] Dengue prevalence among blood donors will depict a good picture of dengue in the population in general. It would come in handy in the future to implement screening, vaccination policies, and probing the utility of other control measures like pathogen inactivation or any other viral inactivation technique if found essential.

Methodology

This was a cross-sectional study performed on samples collected for a period of 1 year starting from February 2019 to January 2020 among the blood donors who attended the Department of Transfusion Medicine of a tertiary care teaching institute for blood donation.

Sample size and Sampling: Assuming a prevalence of 1.5% (range 1%–2%) with a 95% confidence level and a relative precision of 50%, the sample size was calculated to be a minimum of 880 using Daniel's formula. Convenient sampling was used for inclusion in the study. Any three samples, one from each session of the day, namely before Tea, i.e., 11:00 am, before Lunch, i.e., up to 1:00 pm, and post-lunch, were randomly chosen daily for 5 days of the week from Monday to Friday.

Sample collection and storage: 5 ml of venous whole blood was collected from the blood donor in a sterile plain tube for transfusion transmissible infection (TTI) testing as per the departmental Standard Operating Procedure. The blood was then allowed to clot. Samples were centrifuged after that at 3000 rpm for 3 min for separating serum. Then, the separated serum was used for TTI testing, and once all the tests were done, the remaining serum of non-reactive samples was aliquoted into small vials and kept in deep freezers at -30° . The enzyme-linked immunosorbent assay (ELISA) for Dengue NS1 was run after collecting about 75–80 samples, i.e., roughly 5–6 weeks. On the day of testing, all the reagents and specimen were brought to room temperature before use, and thawing was rarely required.

Dengue NS1 Antigen testing: Dengue NS1 Antigen testing using ELISA using QUALISA Dengue NS1

Antigen kits manufactured by Qualpro Diagnostics (a division of Tulip Diagnostics Pvt Ltd), Goa. All the tests were performed as per the manufacturer's instructions in the kit. Samples with absorbance values equal to or greater than the cut-off value were considered reactive. Appropriate controls from the kits and the in-house controls prepared from positive samples were added in each run.

Data collection

Data regarding clinical, epidemiological, and demographic characteristics were collected from the donor questionnaire and records. The test results were entered along with other details in the Microsoft Excel sheet and analyzed.

Ethical clearance was obtained from the Institutional Ethical Committee vide infra letter no JIP/IEC/2018/0196 dated 06/07/2018.

Statistical analysis

The distribution data for the categorical data related to the donor's baseline characteristics, such as age, gender, etc., were expressed as frequencies and percentages. SPSS software version 19.0 (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp) was used for statistical analysis.

Results

We tested 968 blood donor samples. Of these, 9 (0.93%) samples turned out to be positive as per the kit cut-off. Consideration of a 10% gray zone for cut-off would have resulted in an additional two samples being considered reactive. The sociodemographic characters of the study participants are summarized in Table 1 as well as the positives are indicated. The majority (46.3%) of our donors belonged to 18–24 years. Most of them were male (96%) and were students (38.7). Sixty-six percent of our donors were from rural areas. About 42% of our donors were voluntary, and 55.5% were 1st-time donors. The number of positives was skewed toward December, January, and February, as shown in Figure 1. The catchment areas of the donors in our study and the location of positives detected are mapped, as shown in Figure 2.

Discussion

Routine screening is not so feasible for infections which are: (1) Normally not transmitted parenterally, but the possibility of high levels of the infectious agent in an infected donor that can be transmitted if the donor donates during this time, (2) Rarely transmitted that too at significantly lower levels than the prevalence or incidence of the particular infection in the population

and (3) Infections that are frequently transmitted but rarely causing any clinical disease in the recipient.^[5]

Even if they do have screening tests for such infections, they may just be used for diagnostic purposes in symptomatic individuals, and such tests may not be appropriate for the screening of blood donors. The fact that almost 70% of DENV can persist in the donor blood for about 7 days in an acutely infected donor who tends to donate blood during this asymptomatic phase can indeed be a DENV carrier.^[6]

In our tertiary care setup, all the donors accepted for blood donation had no symptoms/signs of dengue as per WHO guidelines, such as fever, headache, retro-orbital pain, myalgia, rashes, or any bleeding manifestations.^[7,8] Our study and similar studies preceded ours are of significance as emerging pathogens, including flaviviruses, usually present asymptomatic forms in healthy individuals and clinically evident in transfusion recipients or patients.^[9]

Comparison of seroprevalence of other similar studies is summarized in Table 2.^[10-12] In this study, both voluntary and replacement donors were included as ours is a 2300-bedded tertiary care hospital that needs to cater to a large patient population more than what the voluntary donations alone can suffice.

Prevalence was higher in 1st-time compared to repeat donors. The seroprevalence of other TTIs decreases with increasing donations because repeat donors are usually better informed about high-risk behaviors due to pre-donation counseling multiple times and less likely to engage in such high-risk activities suddenly. Furthermore, such donors are usually listed negative in the record of their previous donations, and most centers recruit donors after checking their old records.^[13] However, it is not clear whether this holds good for dengue as well, but similar findings were found in a study in Yunnan province, China.^[14]

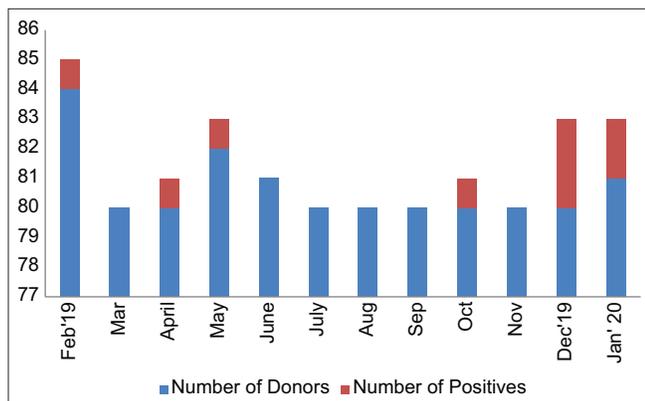


Figure 1: Month-wise distribution of the number of samples tested and positivity noted

A study was done on 910 Saudi blood donors by Ashshi et al., where they found 5.3% seropositivity for Dengue NS1 antigen suggesting asymptomatic viremia in those donors.^[7] This reduction in seropositivity in our study compared to this particular study with a lesser number of donor populations than ours could be attributed to several factors such as selecting random samples wherein we could have missed other potentially viremia donor samples. The test is done probably in the later phase of viremia, where the NS1 antigen detection levels would have been relatively low to be detected by the testing methods, stringent donor selection criteria being followed in our blood bank setup.

In Puducherry, the monsoon is typically from July to September. The months following or the post-monsoon months, usually October to December, extending to January end or early February months, are the ones that have subnormal rainfall with high humidity. A previous study has studied the occurrence of dengue fever in relation to climate variables in Puducherry, wherein a maximum number of cases were recorded during October to December with a peak during November and a gradual decline after that.^[15] Usually, the vector *Aedes aegypti* is shown to have two types of breeding foci; primary and secondary. Usually, the primary foci are formed during the heavy rainfall in the monsoon and pre-monsoon. During the post-monsoon, when the temperature decreases, leading to decreased evaporation, the secondary reservoirs are formed due to stacked water and rampant secondary breeding foci, which cause increased cases during this period.^[16] Similar patterns

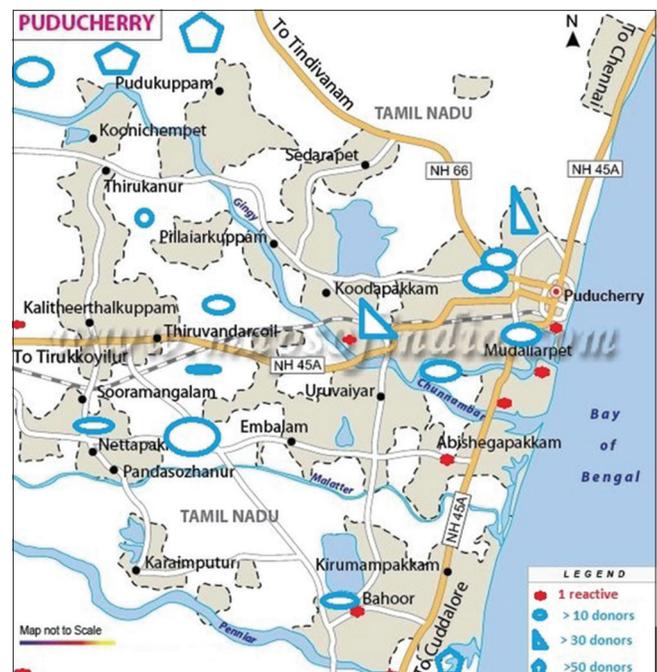


Figure 2: The catchment areas of the donors in our study and location of positives detected

Table 1: Sociodemographic factors of the study population and the dengue positive donors

Sociodemographic characteristics	Categories	Frequency	
		Total donors tested (n=968), n (%)	Number of positives (n=9)
Age (years)	18-24	448 (46.3)	5
	25-34	349 (36.1)	4
	35-44	125 (12.9)	0
	45-54	44 (4.5)	0
	>55	2 (0.2)	0
Sex	Male	929 (96)	8
	Female	39 (4)	1
Occupation	Professional	43 (4.4)	0
	Semi-professional	179 (18.5)	1
	Clerical/shop keeper/farm	114 (11.8)	0
	Skilled	145 (15)	2
	Semi-skilled	47 (4.9)	1
	Unskilled	65 (6.7)	0
Education	Unemployed/students	375 (38.7)	5
	No formal education	8 (0.8)	0
	Primary (up to Class 5)	7 (0.7)	0
	Middle (Class 5-10)	68 (7)	1
	High school	123 (12.7)	2
	Diploma/intermediate (Class 11-12)	218 (22.5)	2
Marital status	Graduation	453 (46.8)	4
	Postgraduation/professional education	91 (9.4)	0
Blood group	Single (never married)	664 (70.2)	6
	Married	288 (29.8)	3
Type of donors	A	172 (17.8)	1
	B	319 (33)	3
	O	410 (42.4)	3
	AB	67 (6.9)	2
Type of donation	Voluntary	405 (41.8)	1
	Replacement	563 (58.2)	8
Residence	First time	537 (55.5)	7
	Repeat	431 (44.5)	2
	Urban	329 (34)	3
	Rural	639 (66)	6

Table 2: Comparison of dengue prevalence from previously published studies

Authors	Study population (n)	Location	Prevalence (%)		
			IgG	IgM	NS1 Antigenemia
Chitra <i>et al.</i> ^[4]	110	Chennai	91.8	0	0
Ashshi <i>et al.</i> ^[7]	910	Saudi Arabia	39	5.5	5.5 (by PCR)
Kulkarni <i>et al.</i> ^[10]	520	Pune	NR	6	0.58
Ranjan <i>et al.</i> ^[11]	200	New Delhi	58	13.5	0 (by PCR)
Jain <i>et al.</i> ^[12]	369	Rishikesh	14.9	NR	0.54
Mangwana <i>et al.</i> ^[26]	1709	New Delhi	NR	NR	0
Current study	968	Puducherry	NR	NR	0.93

PCR=Polymerase chain reaction, NR= Not Reported

have been shown in studies from Andhra Pradesh and Delhi.^[17,18] The postmonsoon period coinciding with post-monsoon has been shown in studies from Bangladesh and Brasil as well.^[19,20]

The peak dengue season in that particular area needs to be kept in mind as it manifests seasonally. Blood banks can use such relatively inexpensive, rapid, and quite sensitive screening methods for routine screening of

donors during the peak rainy seasons/post-monsoon seasons during which the cases of dengue are at an all-time high compared to other months of the year as found in other studies.^[21,22]

Differences in the prevalence among occupational, educational, and ethnic groups are known to some extent. Farmers, residents of rural and lower education-level donors are known to have a higher prevalence and

are known to have something to do with their living environment. DENV is mainly transmitted by the *Aedes Aegyptus*, commonly found in residential areas wherein containers are stagnant water tanks, basins, discarded tires, bamboo tubes, tree holes, and stone pools etc., are found.^[14]

India roughly collects and transfuses about 11–12 million units annually.^[23] About 1% of them being DENV positive in the peak season alone can contribute roughly 30,000–40,000 cases. It should also be noted that roughly only 20% of RNA-positive donations can be picked up by NS1 antigen testing.^[24] Hence, the actual figures may be much worse than this. IgG is not helpful as a screening test for dengue, whereas the IgM alone would fail to help identify the asymptomatic cases or impact the transmission by blood necessarily.^[25] Hence, screening for NS1 antigenemia would be a feasible option in extensive screening compared to detection RNA, which can be much more technically commanding and require more robust infrastructure.

The notable strength of the study is the considerably large number of donors recruited and spread uniformly over all the months of the year. The limitation of the study is that the reactive samples did not undergo repeat testing and were not subjected to more sensitive methods for confirmation of true positives.

Future work may preferably focus on the prospective evaluation of NS1 antigen in all the asymptomatic donors coming for blood donation during peak rainy/post-monsoon seasons. Recognising the blood donation from donors who are residing, especially in endemic areas, as one of the risk factors for dengue transmission in patients may be considered.

Transfusion transmitted infections continue to be a significant threat to the provision of safe blood by transfusion services. Sero-epidemiologic studies are instrumental in understanding the real burden posed by TTIs, in this case, the infection being dengue. In the present study, the overall seroprevalence of Dengue NS1 antigenemia among apparently healthy donors was 0.9% out of 968 blood donor samples tested in the population studied. This should be taken as a pointer of a sizeable proportion of the population in the Southern parts of India being asymptomatic DENV carriers. Follow-up of transfused individuals should go hand in hand in future studies on donor population to see whether transfusion-transmitted dengue is an actual risk. More extensive studies are required to assess the risk of dengue infection in donors and transfusion-transmitted dengue. Such extensive population studies can give more substantial evidence

to be utilized for future donor policies adopted in blood banks.

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Conflicts of interest

There are no conflicts of interest.

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